CLAIMS

1. Method of screening the operating conditions of a coupling reaction of at least two functional groups, which comprises the following steps:

i) reacting together at least two compounds:

- a first compound of formula E_1-X_1-G_1 in which G_1 represents a first of said at least two functional groups, X_1 represents a covalent bond or a first spacer group, while E_1 represents the residue of a first molecule M_1 for which a first specific antibody AC_1 is available, and
- a second compound of formula E_2-X_2-G_2 in which G_2 represents a second of said at least two functional groups, X_2 represents a covalent bond or a second spacer group, which may be identical to or different from X_1, while E_2 represents either the residue of a second molecule M_2 that is different from M_1 and for which a second specific antibody AC_2 is available, or a group capable of forming at least one covalent bond with the antibody AC_1 in the presence of a coupling agent;

said at least two compounds being reacted in solution in a solvent and under predetermined operating conditions, at least one of which is a candidate operating condition, in order to obtain a reaction medium and the formation, in this medium, of a compound Z comprising the chain E_1-X_1-G_1-G_2-X_2-E_2 in which X_1, X_2, E_1 and E_2 have the same meaning as above, while G_1-G_2

represents the group of atoms resulting from the coupling of said at least two functional groups;

ii) determining the concentration of compound Z in the reaction medium at a predetermined reaction time t, by means of at least one immunoassay using at least the antibody AC_1; and

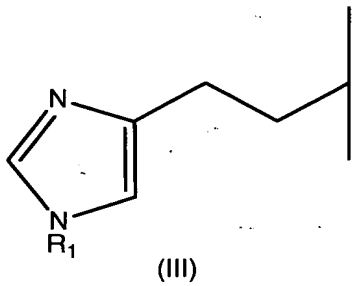
iii) evaluating the effects of the candidate operating condition(s) on said coupling reaction using the concentration of compound Z thus determined.

2. Method according to Claim 1, in which the coupling reaction is chosen from the group consisting of esterification reactions, amidation reactions, aldolization and nitroaldolization reactions, the Heck reaction, the Baylis-Hillman reaction, the Michael reaction, metathesis reactions, the Diels-Alder reaction, the Sonogashira reaction, the Suzuki reaction, the Kumada reaction, the Stille reaction, the Hiyama reaction, the Liebeskind-Srogl reaction, the Mannich reaction, the Hantzsch reaction, the reaction of Bossio et *al.*, the Ugi reaction, and variants thereof.

3. Method according to Claim 1 or Claim 2, in which E_1 or E_2 represents the histamine residue.

4. Method according to Claim 1 or Claim 2, in which E_1 or E_2 represents the homovanillic acid residue.

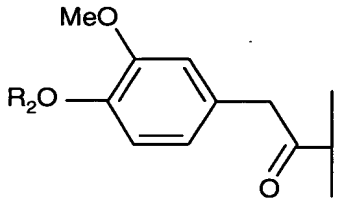
5. Method according to Claim 3, in which E_1 or E_2 corresponds to formula (III) below:



(III)

in which R_1 represents a hydrogen atom or a protective group.

6. Method according to Claim 5, in which E_1 or E_2 corresponds to formula (IV) below:



(IV)

in which R_2 represents a hydrogen atom or a protective group.

7. Method according to Claim 1 or Claim 2, in which E_2 represents a group chosen from amine, carboxylic acid, aldehyde, thiol, phenol, alkenyl and azide groups, and photoactivatable groups.

8. Method according to Claim 7, in which E_2 represents an amine or thiol group.

9. Method according to any one of Claims 1 to 8, in which said at least one immunoassay for the compound Z is a solid-phase assay.

10. Method according to any one of Claims 1 to 6, in which, since E_2 corresponds to the residue of a molecule M_2, step ii) comprises the following steps:

a_1) bringing the reaction medium obtained at reaction time t into contact with a solid phase on which the first antibody AC_1 is immobilized, so as to obtain the attachment of the compound Z on this solid phase by immunobinding between this antibody and the residue E_1 of this compound;

b_1) bringing the solid phase into contact with a conjugate comprising the second antibody AC_2 coupled to a label, so as to obtain the attachment of this conjugate to this solid phase by immunobinding between the second antibody AC_2 and the residue E_2 of the compound Z attached to said solid phase;

c_1) measuring the amount of conjugate attached to the solid phase by means of the label coupled to the antibody AC_2; and

d_1) determining, on a standard range, the concentration of the compound Z in the reaction medium at said time t, from the amount of conjugate thus measured;

said step ii) also comprising one or more operations consisting in washing the solid phase, between steps a_1) and b_1), and between steps b_1) and c_1).

11. Method according to any one of Claims 1, 2, 7 or 8, in which, since E_2 corresponds to a group capable of forming at least one covalent bond with the first antibody AC_1, step ii) comprises the following steps:

a_2) bringing the reaction medium obtained at reaction time t into contact with a solid phase on which the first antibody AC_1 is immobilized, so as to obtain the attachment of the compound Z to this solid phase by immunobinding between this antibody and the residue E_1 of this compound;

b_2) reacting a coupling agent with the first antibody AC_1 immobilized on the solid phase and the group E_2 of the compound Z attached to this solid phase, so as to obtain the formation of one or more covalent bonds between this antibody and this group;

c_2) denaturing the immunobond which exists between the first antibody AC_1 immobilized on the solid phase and the residue E_2 of the compound Z attached to this solid phase, so as to release this residue from this solid phase;

d_2) bringing the solid phase into contact with a conjugate comprising the first antibody AC_1 coupled to a label, so as to obtain the attachment of this conjugate to this solid phase by immunobinding between said antibody and the residue E_1 of the compound E_1-X-G_1-G_2-Y-E_2 thus released;

e_2) measuring the amount of conjugate attached to the solid phase by means of the label coupled to the antibody AC_1; and

f_2) determining, on a standard range, the concentration of compound Z in the reaction medium at said time t, from the amount of conjugate thus measured;

said step ii) also comprising one or more operations consisting in washing the solid phase, between steps a_2) and b_2), b_2) and c_2), c_2) and d_2), and between steps d_2) and e_2).

12. Method according to any one of the preceding claims, in which the first antibody AC_1 is a monoclonal antibody.

13. Method according to any one of Claims 1 to 6 or 10, in which the second antibody AC_2 is a monoclonal antibody.

14. Method according to any one of the preceding claims, in which the solid phase is the wall of a well of a microtitration plate onto which the first antibody AC_1 is adsorbed.

15. Method according to Claim 10 or Claim 11, in which the label is an enzyme, preferably acetylcholine esterase.

16. Method according to any one of the preceding claims, which comprises an operation

consisting of dilution of the reaction medium between steps i) and ii).

17. Method according to any one of the preceding claims, in which the yield of the coupling reaction is determined from the concentration of compound Z in the reaction medium.

18. Method according to any one of the preceding claims, in which the coupling reaction consists in coupling 2, 3 or 4 functional groups.

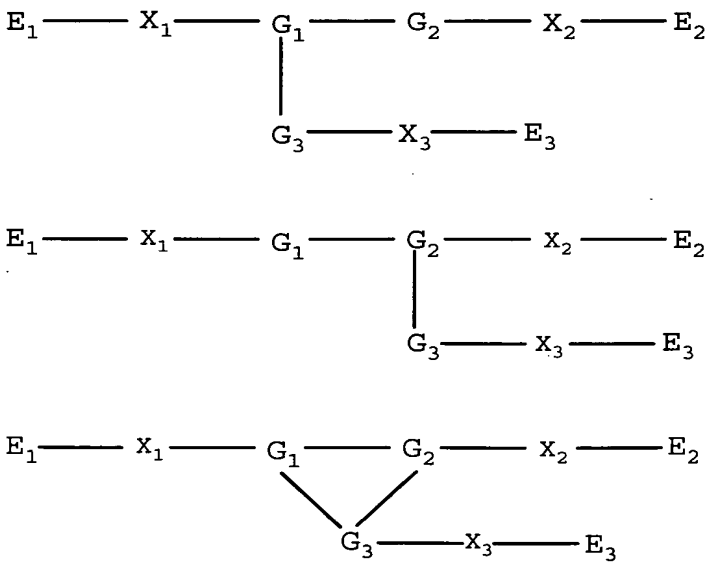
19. Method according to Claim 18, in which the coupling reaction consists in coupling two functional groups G_1 and G_2, and in which:

- in step i), the compounds of formulae E_1-X_1-G_1 and E_2-X_2-G_2 are reacted together so as to obtain the formation, in the reaction medium, of a compound Z which corresponds to the formula E_1-X_1-G_1-G_2-X_2-E_2 in which X_1, X_2, E_1 and E_2 have the same meaning as above and G_1-G_2 represents the group of atoms resulting from the coupling between said functional groups G_1 and G_2; while

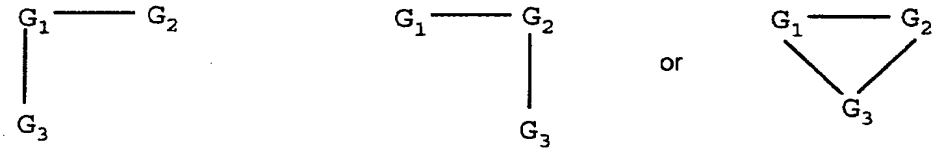
- in step ii), the concentration of compound Z in the reaction medium is determined by means of a single immunoassay.

20. Method according to Claim 18, in which the coupling reaction consists in coupling three functional groups G_1, G_2 and G_3, and in which:

– in step i), the compounds of formulae E_1-X_1-G_1 and E_2-X_2-G_2 are reacted with a third compound of formula E_3-X_3-G_3 in which X_3 represents a covalent bond or a third spacer group, which may be identical to or different from X_1 and/or X_2, while E_3 represents either the residue of a third molecule M_3 which is different from M_1 and from M_2 and for which a third specific antibody AC_3 is available, or a group capable of forming a covalent bond with the antibody AC_1 in the presence of a coupling agent on the condition, however, that E_2 does not already represent such a group, so as to obtain the formation, in the reaction medium, of a compound Z corresponding to one of the formulae below:



in which X_1, X_2, X_3, E_1, E_2 and E_3 have the same meaning as above, and

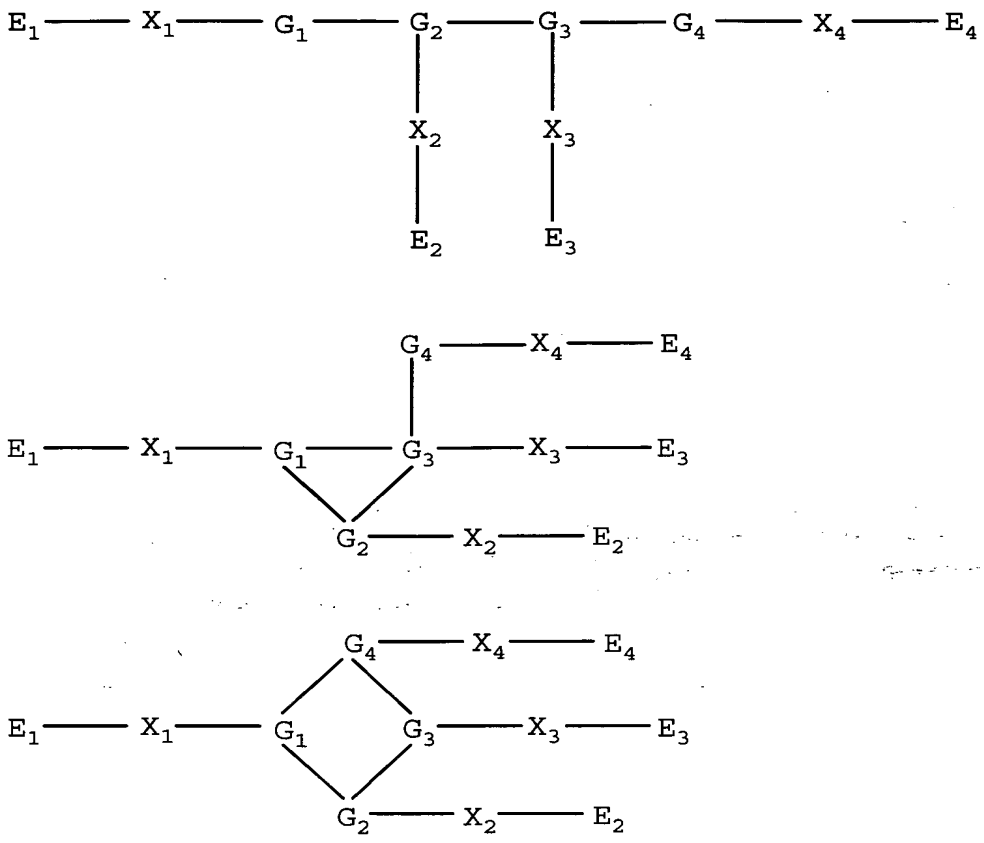


represents the group of atoms resulting from the coupling of said functional groups G_1, G_2 and G_3; while

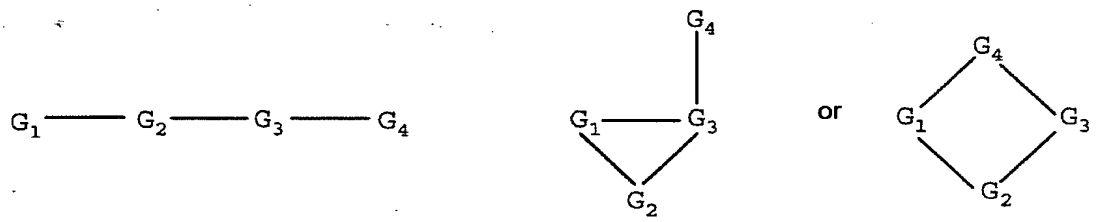
- in step ii), the concentration of compound Z in the reaction medium is determined by means of two different immunoassays.

21. Method according to Claim 18, in which the coupling reaction consists in coupling four functional groups G_1, G_2, G_3 and G_4, and in which:

- in step i), the compounds of formula E_1-X_1-G_1 and E_2-X_2-G_2 are reacted with a third compound of formula E_3-X_3-G_3 as defined above and a fourth compound of formula E_4-X_4-G_4 in which X_4 represents a covalent bond or a fourth spacer group, which may be identical to or different from X_1, X_2 and/or X_3, while E_4 represents either the residue of a third molecule M_4 which is different from M_1, from M_2 and from M_3 and for which a fourth specific antibody AC_4 is available, or a group capable of forming a covalent bond with the antibody AC_1 in the presence of a coupling agent, on the condition, however, that E_2 and E_3 do not already represent such a group, so as to obtain the formation, in the reaction medium, of a compound Z corresponding to one of the formulae below:



in which X_1, X_2, X_3, X_4, E_1, E_2, E_3 and E_4 have the same meaning as above, and



represents the group of atoms resulting from the coupling of said functional groups G_1, G_2, G_3 and G_4; while

- in step ii), the concentration of compound Z in the reaction medium is determined by means of three different immunoassays.

22. Method according to any one of the preceding claims, in which the candidate operating condition(s) is(are) chosen from the group consisting of solvents, catalysts, temperature levels, pressure levels, the use of ultrasound, concentrations, stoichiometric ratios, reaction times and combinations thereof.

23. Method according to any one of the preceding claims, in which the candidate operating condition(s) is(are) catalysts.

24. Kit for carrying out a method of screening the operating conditions of a coupling reaction of at least two functional groups, which comprises suitable amounts:

– of at least two compounds intended to react together:

• a first compound of formula E_1-X_1-G_1 in which G_1 represents a first of said at least two functional groups, X_1 represents a covalent bond or a first spacer group and E_1 represents the residue of a first molecule M_1; and

• a second compound of formula E_2-X_2-G_2 in which G_2 represents a second of said at least two functional groups, X_2 represents a covalent bond or a second spacer group, which may be identical to or different from X_1, and E_2 represents the residue of a second molecule M_2 which is different from M_1;

– of at least two antibodies:

• a first antibody AC_1 specific for the first molecule M_1, this antibody being optionally attached to a plurality of solid phases; and

• a second antibody AC_2 specific for the second molecule M_2, this antibody being coupled to a label;

– of a compound Z comprising the chain E_1-X_1-G_1-G_2-X_2-E_2 in which X_1, X_2, E_1 and E_2 have the same meaning as above, while G_1-G_2 represents the group of atoms resulting from the coupling of said at least two functional groups; and, optionally:

– of a reagent for visualizing the label, for example a substrate if the label is an enzyme; and

– of suitably chosen buffers.

25. Kit for carrying out a method of screening the operating conditions of a coupling reaction of at least two functional groups, which comprises suitable amounts:

– of at least two compounds intended to react together:

• a first compound of formula E_1-X_1-G_1 in which G_1 represents a first of said at least two functional groups, X_1 represents a covalent bond or a first spacer group and E_1 represents the residue of a first molecule M_1; and

• a second compound of formula E_2-X_2-G_2 in which G_2 represents a second of said at least two functional groups, X_2 represents a covalent bond or a second spacer group, that may be identical to or different from X_1, and E_2 represents a group capable of forming one or more covalent bonds with an antibody specific

for the molecule M_1 in the presence of a coupling agent;

– of at least one antibody, this antibody being said antibody specific for the molecule M_1;

– of a conjugate comprising said antibody specific for the molecule M_1 coupled to a label;

– of a compound Z comprising the chain E_1-X_1-G_1-G_2-X_2-E_2 in which X_1, X_2, E_1 and E_2 have the same meaning as above, while G_1-G_2 represents the group of atoms resulting from the coupling of said at least two functional groups; and, optionally:

– of a reagent for visualizing the label,

– of a coupling agent,

– of a reagent capable of denaturing an immunobond, and

– of suitably chosen buffers.

26. Use of a screening method according to any one of Claims 1 to 23 or of a kit according to Claim 24 or Claim 25, for the screening, in particular the "high-throughput" screening, of catalysts that are useful in a coupling reaction between two functional groups.